PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PCT0027	**FOR FURTHER ACTION** See Form PCT/IPEA/416	
International application No. PCT/IN2004/000394	International filing date *(day/month/year)* 22.12.2004	Priority date *(day/month/year)* 23.12.2003
International Patent Classification (IPC) or national classification and IPC G01N33/543		
Applicant MAHARASHTRA HYBRID SEEDS COMPANY LIMITED et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 7 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 3 sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☒ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☒	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
16.08.2005	24.11.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Diez Schlereth, D Telephone No. +49 89 2399-7488

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/IN2004/000394

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

   Description, Pages

   1-15 as originally filed

   Claims, Numbers

   1-20 as originally filed

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☒ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☒ the claims, Nos. 1-19 (filed with letter of 22.07.05)
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☐ claims Nos.

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos.

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☒ See separate sheet for further details

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-19
	No:	Claims	20
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-20
Industrial applicability (IA)	Yes:	Claims	1-20
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

item III

This report has been established as if the amendments filed with the letter of 22.07.05 (claims 1-19) had not been made because said amendments introduce subject-matter which was not unambiguously disclosed in the text of the application as originally filed (Rule 70.2 (c) PCT), the reasons being as follows:

The scope of independent claims 1, 16 and 18-19 results from an unallowable generalization of a single embodiment (see claims 1, 16 and 19-20 as originally filed and p. 3, l. 24 to p. 4, l. 15, p. 5, l. 23 to p. 6, l. 24 of the description), which introduces subject-matter that was not unambiguously disclosed in the application as originally filed.

Therefore, the present international preliminary examination report concerns the set of claims 1-20 as originally filed.

item V

1.) Reference is made to the following documents:

D1: EP-A-1 304 574
D2: WO-A-02/052263
D3: WO-A-02/090983
D4: WO-A-02/14868
D5: JP-A-02 161 357
D6: JP-A-08 015 261
D7: JP-A-63 111 467
D8: R. I. Vázquez et al (1996) J. Immunol. Meth. 196, 33
D9: F. E. Ahmed (2002) TRENDS in Biotechnol. 20, 215-223

2.) The subject-matter of claim 20 is not novel within the sense of Art. 33 (2) PCT, for the following reasons:

D1 discloses a solid support (and a kit) for detection of CMV in a sample using an ELISA assay (see examples 2 and 5). Analogously, D2 (see p. 22-23) and D3 (example 4) also

disclose kits for detection of proteins in a sample using an ELISA assay. In D1 and D2, the preparation of the ELISA plates involves physical adsorption of the capture antigens (or antibodies) in the presence of a pH-stabilized buffer, washing, incubation with a blocking agent (BSA) and DRYING (applying vacuum) the immobilized material. The coating process of D3 is similar, but the immobilized material is air-dried. After that, the wells of the plate are provided with further reagents, such as an enzyme-labeled conjugate and standard solutions, which are LYOPHILIZED (freeze-dried) in order to obtain a ready-to-use plate (see D3, example 1). D4 (see p. 12) discloses a method for preparing ELISA plates involving deposition of first capture antibody, second analyte-specific antibody and enzyme-antibody conjugate and using microwaves for drying/stabilizing each layer. Further solid supports for carrying out immunoassay are disclosed in D5-D7 (see abstracts). The coating processes disclosed in D5-D7 involve the use of stabilizers and freeze-drying. D8 (see abstract) and D9 (p. 217-218) disclose ELISA plates for detection of Cry proteins and for detection of EPSPS.

I would appear that anyone of D1-D9 anticipates the subject-matter of claim 20.

3.) The subject-matter of claim 19 is considered to be novel, but not inventive within the sense of Art. 33 (3) PCT, for the following reasons: the kit of claim 19 differs from the closest state of the art (anyone of D1, D2 or D3) in that it comprises an instruction manual. This difference, however, is considered to be an obvious alternative of the kit of D3 (or D1, or D2), which falls within the routine practice in this technical field and which does not result in any unexpected technical effect.

4.) The subject-matter of claims 1-18 is considered to be novel, but not inventive within the sense of Art. 33 (3) PCT, for the following reasons:

The method of claim 1 differs from the method of D3 (closest state of the art) in that after step d) (in which the wells are dry and coated with a primary antibody), an appropriate second antibody specific for the analyte is added to the wells together with the (third) enzyme-antibody (detection) conjugate.

In the light of D4, however, it would appear that the use of second analyte-specific antibody for providing a verification that the captured analyte is indeed the seeked one is an obvious alternative, which belongs to the routine practice in this technical field, and which does not

seem to result in any unexpected technical effect.

In the light of the known prior art (D1-D9), the subject-matter of dependent claims 2-15 seems to relate to obvious alternatives of the method of claim 1, which belong to the routine practice in this technical field. Analogous arguments apply for the subject-matter of claims 16-18.

item VIII

Claims 19-20 do not meet the requirements of Art. 6 PCT because the subject-matter for which protection is sought is not defined at all (the solid support may be anyone used in a common immunoassay). For this reason, it is not possible to carry out a meaningful search embracing the whole scope of the claims.

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I / We claim

1. A method for preparing ready-to-use solid support for rapid ELISA, wherein the said method comprises addition of first monoclonal antibody, washing with buffer to remove unbound monoclonal antibody adding a stabilizer, removing excess stabilizer, air-drying of the bound stablilizer, addition of an appropriate second antibody and enzyme linked conjugate as third antibody together dissolved in buffer, lyophilising the said protein mixture and storing in a sealed package at a specified temperature.

2. A method as claimed in claim 1, wherein the first monoclonal antibody is raised against the protein/antigen to be detected.

3. A method as claimed in claim 1, wherein the first monoclonal antibody used is selected from a group consisting of monoclonal antibodies raised against Cry proteins and monoclonal antibodies against 5-enolpyruvylshikimate-3-phosphate synthase, wherein Cry protein is preferably selected from Cry1Ab, Cry1Ac Cry2Ab, Cry 9A, Cry 9B and Cry 9C.

4 A method as claimed in claim 1, wherein the buffer used for washing is phosphate buffer saline having a pH in the range of 6.8-7.2.

5. A method as claimed in claim 1, wherein buffer used for dissolving second and third antibody is selected from a group consisting of carbonate buffer and phosphate buffer, having pH in the range of 9.0-9.8.

6. A method as claimed in claim 1, wherein the stabilizer used is selected from a group consisting of Phosphate Buffered Saline, Fish Gelatin and Glycerol mixture and a Tris-buffer, Fish Gelatin and Glycerol mixture.

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AMENDED SHEET (ARTICLE 19)

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7. A method as claimed in claim 1, wherein the drying method used is either freeze drying or lyophilization.

8. A method as claimed in claim 1, wherein the blocking agent used is selected from the group consisting of ovalbumin, bovine serum albumin, bovine nonfat milk powder, casein, fish gelatin, porcine gelatin and lambda-carrageenan.

9. A method as claimed in claim 1, wherein the solid support used is selected from the group consisting of ELISA plate and microwell plate.

10. A method as claimed in claim 1, wherein the material for the solid support used is either polystyrene or polypropylene.

11. A method as claimed in claim 9, wherein the solid support is made of polystyrene.

12. A method as claimed in claim 1, wherein second antibody used is polyclonal antibody IgG raised against protein/antigen to be detected.

13. A method as claimed in claim 1, wherein second antibody used is polyclonal antibody IgG raised against corresponding Cry protein or IgG raised against 5-enolpyruvylshikimate-3-phosphate synthase.

14. A method as claimed in claim 1, wherein third antibody used is selected from the group consisting of polyclonal whole IgG conjugated to an enzyme, wherein whole IgG may be obtained from class Mammalia or class Aves.

15. A method as claimed in claim 14, wherein the enzyme used is selected from a group consisting of alkaline phosphatase and horseradish peroxidase

AMENDED SHEET (ARTICLE 19)

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16. A rapid method for performing ELISA using ready-to-use solid support of claim 1 said method comprising steps of reconstituting the ready to use plates by adding appropriate amount of distilled water, adding test samples containing antigen/protein are dissolved in a suitable buffer, washing the plate after incubating for a required time period, followed by washing with suitable buffer, adding to the plate required chemical substrate and detecting for the presence of the antigen by measuring absorbance at a suitable wavelength.

17 A method as claimed in claim 16, wherein the chemical substrate is selected from the group consisting of para-nitrophenol phosphate, Nitro Blue Tetrazolium/5-Bromo-4-Chloro-3-Indolyl Phosphate, 2,2'-Azino-bis (3-Ethylbenz-thiazoline-6-Sulfonic Acid), o-Phenylenediamine, 3,3'-5,5'-Tetramethylbenzidine, o-Dianisidine and 5-Aminosalicylic Acid.

18. An immunoassay kit comprising of ready to use solid support of claim 1 for rapid ELISA .

19. A ready-to-use solid support of claim 1 for detection of protein or antigen

AMENDED SHEET (ARTICLE 19).